Molecular Genetic Characterization of *Sweet potato virus G* (SPVG) Isolates from Areas of the Pacific Ocean and Southern Africa

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ABSTRACT

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Sweet potato virus G (SPVG, genus *Potyvirus*, family *Potyviridae*) was detected in sweetpotato (*Ipomoea batatas*) storage roots sold in the local markets and storage roots or cuttings sampled directly from farmers' fields. Using serological and molecular methods, the virus was detected for the first time in Java, New Zealand, Hawaii, Tahiti, Tubuai, Easter Island, Zimbabwe, and South Africa, and also in an imported storage root under post-entry quarantine conditions in Western Australia. In some specimens, SPVG was detected in mixed infection with *Sweet potato feathery mottle virus* (genus *Potyvirus*). The coat protein (CP) encoding sequences of SPVG were analyzed for 11 plants from each of the aforementioned locations and compared with the CP sequences of 12 previously characterized isolates from China, Egypt, Ethiopia, Spain, Peru, and the continental United States. The nucleotide sequence identities of all SPVG isolates ranged from 79 to 100%, and amino acid identities ranged from 89 to 100%. Isolates of the same strain of SPVG had nucleotide and amino acid sequence identities from 97 to 100% and 96 to 100%, respectively, and were found in sweetpotatoes from all countries sampled except Peru. Furthermore, a plant from Zimbabwe was co-infected with two clearly different SPVG isolates of this strain. In contrast, three previously characterized isolates from China and Peru were phylogenetically distinct and exhibited <90% nucleotide identity with any other isolate. So far, the highest genetic diversity of SPVG seems to occur among isolates in China. Distribution of SPVG within many sweetpotato growing areas of the world emphasizes the need to determine the economic importance of SPVG.

Sweetpotato (*Ipomoea batatas* Lam.) is the third most important root crop globally after potato and cassava. It is grown in all subtropical and tropical climatic regions of the world (15). Based on genetic diversity studies, the center of origin of sweetpotato is believed to be Mexico and Central America (42), although the oldest indications of cultivated sweetpotatoes come from Peru (32). In the islands of the Pacific Ocean, long before the arrival of European colonizers, sweetpotatoes were widely grown by Polynesian inhabitants. The oldest archeological records of sweetpotato in this region are from the Cook Islands of

Central Polynesia (A.D. 1000 to 1100), Hawaii (A.D. 1290 to 1430), and Easter Island (ca. A.D. 1500) (32). Central Polynesia (Tuamotu, Marquesas, and Society islands) may be the likely area of original arrival of sweetpotatoes in Oceania coming from the coast of Ecuador, Peru, or northern Chile (10,32). In Australasia, sweetpotatoes were first introduced to New Zealand about 800 years ago by Maori settlers from Polynesia. Before European colonization, the crop (known as kumara) was cultivated widely by the Maoris as a staple carbohydrate food source. Although its use declined following the introduction of alternative staple carbohydrate crops, such as potato and maize (29), it is still regarded as an important crop there with 1,400 ha grown annually. In Australia, sweetpotatoes were introduced less than 200 years ago following European colonization, although Australian aboriginals apparently had harvested native yams (*I. polpha* and *I. costata*) for millennia. Sweetpotatoes were

possibly introduced to the Philippines and India by Spanish voyagers in the sixteenth century (10), but it seems unlikely that these were the first introductions to these locations.

Viruses are the most serious pathogens limiting sweetpotato production worldwide (30), but relatively little is known about the occurrence of viruses in sweetpotatoes grown in Oceania. Sweet potato caulimovirus (SPCaLV; genus *Caulimovirus*, family *Caulimoviridae*) has been detected in sweetpotato samples from Papua New Guinea, Solomon Islands, Tonga, New Zealand, and Australia, and Sweet potato ringspot virus (SPRSV; a putative member of the genus *Nepovirus*) from Papua New Guinea (5,7). Detections of *Sweet potato feathery mottle virus* (SPFMV; genus *Potyvirus*, family *Potyviridae*) in Fiji, Tonga, and the Solomon Islands are included in the listing of Pearson and Grisoni (34). Fletcher et al. (16) surveyed sweetpotato crops in the Kaipara district of Northland, which produces 80% of the crop in New Zealand. Their serological tests detected the three potyviruses SPFMV, *Sweet potato latent virus*, and the C8 virus that was later named *Sweet potato mild speckling virus* (41); *Sweet potato mild mottle virus* (genus *Ipomovirus*, family *Potyviridae*), Sweet potato chlorotic fleck virus (SPCFV), and C6 virus, another putative member of the genus *Carlavirus* (*Flexiviridae*) (41); *Cucumber mosaic virus* (genus *Cucumovirus*, family *Bromoviridae*) and SPCaLV. Studies of viruses in sweetpotatoes in Australia have revealed the occurrence of SPFMV (17,20,22,37,39), Sweet potato virus 2 (SPV2, genus *Potyvirus*) (37), and SPCFV (22). According to Salazar and Fuentes (35), most of these and many other viruses have been detected in Indonesia, the Philippines, and Papua New Guinea, but the original reports and details of detection methods were not cited.

The species *Sweet potato virus G* (SPVG) is a member of genus *Potyvirus* that has gained relatively little attention so far. It was originally described from China (11,12) and is also known to occur in the

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United States (36). In Africa, SPVG has been detected in sweetpotatoes grown in Egypt (21) and Ethiopia (1), whereas extensive surveys of sweetpotato viruses in the Lake Victoria basin in East Africa and northern parts of South Africa have not detected it (2–4,33,38). The goal of this study was to survey sweetpotatoes grown in Oceania for SPVG and to assess the genetic diversity of the virus. Samples were collected from Java, Hawaii, French Polynesia, New Zealand, and Easter Island. In addition, a few samples of sweetpotato were collected from Zimbabwe and the southernmost part of South Africa. The results revealed that SPVG is geographically much more widely distributed in the world than previously realized and that many isolates from geographically distant locations were identical.

MATERIALS AND METHODS

Plant material. Storage roots of an unknown, purple-skinned landrace of sweetpotato were collected from a commercial planting at Toahotu, in the south of the island of Tahiti, French Polynesia, in September 2004. Similarly, an unnamed purple-skinned landrace was sampled in Mataura on the island of Tubuai that also belongs to French Polynesia, in October 2004. From New Zealand, cuttings from eight sweetpotato cultivars or landraces growing in home gardens in Dargaville in Northland were sampled in November–

December 2004. One of these was cv. Owairaka Red that dominates the sweetpotato industry in New Zealand. It is a selected red-skinned, cream-fleshed mutant of a local ‘Waina’ landrace introduced from Rarotonga in the 1850s (29). On Easter Island, storage roots of four cultivars or landraces of sweetpotato with different skin colors and shapes were purchased from an open farmers’ market at Hanga Roa in March 2005.

Samples from South Africa and Zimbabwe were obtained in April 2005. Two batches of storage roots of the red-skinned local cultivar Borie were purchased separately at a vegetable stall and in a supermarket in Capetown, South Africa. In Zimbabwe, storage roots of two cultivars, one white skinned (Chena) and the other red skinned (Tsvuku), were dug in a field close to Victoria Falls airport. More storage roots of cv. Chena were obtained from a field in Mukhosana nearby.

In January 2007, sweetpotatoes were purchased from a roadside stall at Lembang in Java and at an open farmers’ market at Hilo on the Island of Hawaii. In Java, as sweetpotatoes were uncommon in the markets at this time, only one white-skinned cultivar, Ubi Kayu, was available. The three cultivars from Hawaii were distinguished by the color and shape of the storage roots (Table 1).

Storage roots or cuttings of the sweetpotatoes collected at the above locations were

sent to the University of Helsinki, Finland, where they were planted and grown in an insect-proof greenhouse under the conditions stipulated in the import permit provided by the Finnish plant quarantine authorities. Plants of the indicator host *I. setosa* were grown from seed. Plants were grown in natural daylight under a 16-h photoperiod (light intensity 250 $\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) extended and supplemented when needed with illumination from high pressure sodium lamps (OSRAM E40/ES, 400W) at a temperature of 20/24°C (night/day). The only exception to this was a storage root of cv. Owairaka Red sent from New Zealand to Perth, Western Australia in March 2005. This was grown in a post-entry quarantine glasshouse at South Perth, where eight cuttings propagated from it were each grafted with *I. setosa* scions.

Virus testing. S. Fuentes and L. F. Salazar (International Potato Center – CIP, Lima, Peru) and J. Vetten (Institute for Plant Virology, Braunschweig, Germany) kindly supplied virus-specific polyclonal and monoclonal antibodies, and enzyme conjugates to SPVG, SPFMV, and *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*, family *Closteroviridae*) for use in nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA), double antibody sandwich (DAS) or triple antibody sandwich (TAS) ELISA. Previously described procedures were followed to test samples of sweetpotato or *I. setosa* leaf tissue by NCM-ELISA (17) or DAS-ELISA and TAS-ELISA in microtiter plates (37). For testing *I. setosa* tissue extracts in Australia for SPVG, SPFMV, SPCSV, and six other viruses that infect sweetpotato, polyclonal and monoclonal antibodies were used in DAS-ELISA and TAS-ELISA, respectively. The test procedures followed for the viruses were as described by Tairo et al. (37). The NCM strips spotted with sap from virus-positive and noninfected control plants were supplied by CIP and used to interpret the results of NCM-ELISA. For the microtiter plate ELISAs, absorbance values were recorded at 405 nm ($A_{405\text{nm}}$), and readings at least three times the values of the negative control samples were considered positive.

Owing to relatively high levels of resistance to potyvirus accumulation in many sweetpotato cultivars, virus titers are often low and direct virus detection from sweetpotato may be unreliable using serological tests. Therefore, grafting with *I. setosa*, a nearly universal host for sweetpotato viruses (7), is used to boost virus titers, and leaves of the grafted *I. setosa* are tested for viruses. In addition, graft-inoculation with SPCSV can be used to boost the titers of potyviruses (24,25), including SPVG (26), in sweetpotato plants. Therefore, a few sweetpotato plants were graft-inoculated with SPCSV-Ug (13,27) to increase the possibility of detecting SPVG by serological tests.

Table 1. Isolates of *Sweet potato virus G* (SPVG) characterized in this study and the publicly available sequences of SPVG

Isolate	Origin and sampling year	Cultivar ^a	Accession no.	Reference
3817	Toahotu, Tahiti, 2004	-(PS)	EU220757	This study
3900A	Mataura, Tubuai, 2004	-(PS)	EU220759	This study
CTB3	Capetown, South Africa, 2005	Borie	EU220756	This study
HAWA2	Hilo, Hawaii, 2007	-(WF, PS, IS)	EU220753	This study
HAWB3 ^b	Hilo, Hawaii, 2007	-(OF, PS, OS)	–	This study
HAWC4 ^b	Hilo, Hawaii, 2007	-(WF, WS, ES)	–	This study
JAAC	Lembang, Java, 2007	Ubi Kayu	EU220755	This study
NZ-4	Dargaville, New Zealand, 2004	Owairaka Red	EU220758	This study
PS1B	Hanga Roa, Easter Island, 2005	Tukuhamei	EU220754	This study
SP1, SP2 ^c	Dargaville (New Zealand) via post-entry quarantine in W-Australia, 2005	Owairaka Red	EF514221	This study
ZIMB1C	Victoria Falls, Zimbabwe, 2005	Chena	EU220752	This study
ZIMB1H	Victoria Falls, Zimbabwe, 2005	Chena	EU220751	This study
Ark15	Areka, Ethiopia	Gadissa	Not available	(1)
Henan	Henan, China	Unknown	DQ399861	–
Hua2	Huaral, Peru	Unknown	EU218528	(40)
Sichuan1	Sichuan, China	Xushu #18	EU218531, EU218532	–
Sichuan2	Sichuan, China	Sankang #1	EU216733	(19)
LSU-3	Louisiana, USA	Unknown	AY178990	(36)
LSU-1	Louisiana, USA	Unknown	AY178991	(36)
SPVG-CH	Guangdong, China	Unknown	X76944	(11)
SPVG-CH2	Guangdong, China	Unknown	Z83314	(12)
SPVG-Eg	Egypt	Abces	AJ515380	(21)
SPVGMal02	Spain	Unknown	EF577438	–
SPVG181-3Can07	Spain	Unknown	EF577439	–

^a Cultivar if known, and characteristics of storage roots for unknown cultivars: ES, elongated shape; IS, irregular shape; OF, orange flesh; OS, oval shape; PS, purple skin; WF, white flesh; WS, white skin.

^b The characterized 3′-proximal part of the viral sequence of the isolate was identical to HAWA2.

^c Sequences of the isolates from the two plants SP1 and SP2 of the same cultivar were characterized and found to be identical.

RNA extraction, cDNA synthesis, and PCR amplification. Molecular characterization of the SPVG isolates was done in Finland unless indicated otherwise. For reverse transcription–polymerase chain reaction (RT-PCR) analysis, leaf samples (0.5 g) were frozen in liquid nitrogen and ground with mortar and pestle. Total RNA was extracted using a Trizol-like reagent (8). RNA was diluted to 200 µl with nuclease free water (Sigma-Aldrich, St. Louis, MO, USA) and quantified with a spectrophotometer (Agilent 8452 A, Agilent Technologies). The cDNA synthesis was carried out in a 25-µl volume with 3 µg of total RNA, polyT(25) primer, and *Moloney murine leukemia virus* (MMLV) reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's instructions. The cDNA was diluted 1:10 and used as a template in PCR amplification.

Primers SPVG1-F (5'-TATACCGCGG AAAAGCACCCTACATAGCT-3') and SPVG3-R (5'-TATATGAGTCCACTGA AGGCGAAACTGAAA-3') were designed according to the SPVG sequences available from databases. The primers included restriction sites for *SacII* and *SacI*, respectively, for cloning purposes (underlined). The amplified fragment of 1,285 nucleotides (nt), including primer sequences, consisted of 161 nt of the replicase (NIb) encoding region, the entire coat protein (CP) encoding region (1,068 nt), and 51 nt of the 3'-nontranslated (3'NTR) region.

The PCR (25 µl) consisted of 5 µl of 5 × Phusion GC Reaction buffer (containing 7.5 mM MgCl₂), 0.5 µl of 50 mM MgCl₂, 0.5 µl of 10 mM dNTPs, 2 µl of 10 µM primers, 0.25 µl (0.5 U) Phusion high-fidelity DNA polymerase (Finnzymes, Espoo, Finland), and 2.5 µl of cDNA. The cycling parameters were: 1 cycle at 98°C for 1 min, followed by 40 cycles of 98°C for 10 s, 63°C for 20 s, and 72°C for 45 s, followed by a final extension at 72°C for 10 min. Amplification products were analyzed by agarose gel electrophoresis and visualized under UV-light.

In Australia, symptomatic leaf tissue of *I. setosa* was ground to a fine powder under liquid nitrogen and the RNA extracted with an RNeasy Plant Mini kit (Qiagen, Doncaster, Victoria, Australia) according to the manufacturer's instructions. The entire SPVG CP-encoding region was amplified by RT-PCR using two pairs of primers that generate overlapping products. The forward primer PMB-9 (5'-GGAAGTATCAGAGTATGCACGAG-3') and the reverse primer PMB-10 (5'-GTTCCATTCTCGATGCAC-3') amplify a 719-nt fragment from the 5'-proximal part, whereas primers PMB-11 (5'-GCACCAATGGCAAATGGAAG-3') and PMB-12 (5'-ACACTGAAGGCGAACTG-3') amplify a 688-nt region from the 3'-proximal part of the CP-encoding sequence. RT-PCR was carried out using the GeneAmp

RNA PCR kit (Applied Biosystems, Scoresby, Australia) as suggested by the manufacturer. The RT reaction mixture had a final volume of 10 µl and contained 10 pmol of reverse primer (PMB-10 or PMB-12) and 1 µl of RNA extract. The reaction was incubated at 42°C for 30 min, followed by 96°C for 5 min. For PCR, the volume of the reaction mixture was increased to 50 µl, and 10 pmol of forward primer (PMB-9 or PMB-11) was added. PCR cycling involved one cycle of initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 7 min. PCR products were purified using a Qiagen PCR Purification kit (Qiagen, Doncaster, Victoria, Australia) following the manufacturer's instructions.

Cloning, sequencing, and sequence analysis. In Finland, the PCR amplification products were purified either with isopropanol precipitation or from the gel using a Gel Extraction Kit (Omega Bio-Tek, Lilburn, GA, USA). Prior to ligation, an A-tailing reaction was carried out with the PCR products. The reaction (10 µl) contained 1 µl of 10 mM dATP, 1 µl of 10× optimized DyNAzyme PCR buffer (containing MgCl₂), and 1 U of DyNAzyme II DNA polymerase (Finnzymes). The reaction was carried out at 70°C for 30 min and terminated by transferring the tube to ice. Subsequently, PCR products were ligated to the pGEM-T or pGEM-T Easy Vector (Promega) according to manufacturer's instructions and cloned in *Escherichia coli* (strain DH5α). The plasmids were purified from *E. coli* with GenElute Plasmid Miniprep Kit (Sigma-Aldrich) and sequenced first using M13 primers (Promega) and subsequently nested primers designed according to the viral sequences. Both strands of at least two independent cDNA clones from each SPVG isolate were sequenced. All sequencing was done with Applied Biosystems Dye Terminator (v.3.1) sequencing kit and a capillary sequence analyzer at the Haartman Institute, Sequencing Core Facility, University of Helsinki.

In Australia, RT-PCR products were sequenced directly from both strands in an ABI 3730 DNA Analyzer using BigDye v. 3.1 Terminator chemistry (Applied Biosystems). Raw sequence data were edited with Sequence Scanner V. 1 (Applied Biosystems) and the overlapping fragments concatenated using BioEdit version 5.0.6. (provided by T. Hall, Department of Microbiology, North Carolina State University, Raleigh, USA). Sequence alignments and phylogenetic analysis using the neighbor-joining method were done using the MEGA program (version 3.1) (28). The "showaling" function of EMBOSS (The Finnish IT Center for Science, Espoo, Finland) was used to display the multiple amino acid sequence alignments.

Additional verification of the sequences. The sequence data were verified by additional analyses to confirm that they were accurate and not an artifact arising from PCR, sequencing errors, contamination of samples, or other technical errors. Therefore, in addition to primers designed from SPVG sequences, the samples were also tested with a forward degenerate primer (PVD-2) complementary to the nucleotide sequence coding for the conserved GNNSGQ motif in the NIb region of the *Potyvirus* (18) and the reverse primer (10820R) complementary to the 3' end of the 3'NTR of viruses of the genus *Potyvirus* (39) and which amplify many different potyviruses.

The storage roots from Hawaii and Java were collected 2 years after the other samples, planted in a different greenhouse compartment, and grown in isolation to obtain small shoots and leaves. They were not handled before taking leaf samples for RNA extraction and RT-PCR analysis. The PCR products were cloned and sequenced, as with all previously characterized SPVG isolates. Subsequently, all reagents needed for the work were replaced with fresh stocks; new leaf samples were taken from the same plants; but this time, the PCR products were sequenced directly (without cloning). RNA was also extracted again from stored, frozen leaves of the plants NZ-4 from New Zealand and CTB3 from South Africa, analyzed by RT-PCR, and the PCR products subjected to direct sequencing.

RESULTS

Detection of SPVG in sweetpotatoes.

When grown in the greenhouse in Finland, no apparent symptoms developed in the shoots of the sweetpotatoes from Tahiti, whereas the plants from Tubuai displayed vein clearing and chlorotic spots on leaves. SPVG was not detected in these plants by repeated tests using NCM-ELISA (data not presented), whereas the plants from both sources were positive for SPVG when tested by RT-PCR. However, when the plants were graft-inoculated with SPCSV, the increased titers of SPVG became detectable by NCM-ELISA in the sweetpotato plants from Tahiti but not from Tubuai. All plants were SPFMV-positive when tested by NCM-ELISA (data not shown). The CP-encoding region of SPVG was amplified, cloned, and the sequences characterized for SPVG isolates from one plant of each landrace (3817 from Tahiti and 3900A from Tubuai) (Tables 1 and 2).

The plants grown from storage roots obtained from Easter Island showed no symptoms, but RT-PCR detected SPVG in the leaves of the red-skinned cultivar Tukuhamei (Table 1). The CP-encoding sequence of this isolate (PS1B) was cloned and sequenced.

All plants grown from cuttings from eight sweetpotato cultivars or landraces

from New Zealand tested negative for SPVG in NCM-ELISA, but NCM-ELISA detected SPVG (isolate NZ-4) in scions of *I. setosa* grafted onto vines of one plant of cv. Owairaka Red (Table 1), and this isolate was characterized.

1). The CP sequences of two isolates (ZimblC, ZimblH) were characterized from this plant.

encoding region revealed that the plant from Zimbabwe was co-infected with two clearly different SPVG isolates, ZIMB1C and ZIMB1H (Table 2). Additional clones were amplified and sequenced, which confirmed the result (data not shown). All sequences characterized in this study differed from the previously characterized CP-encoding sequences of isolates Henan, Sichuan1, SPVG-CH, and SPVG-CH2 from China, isolate SPVG-Eg from Egypt, Ark15 from Ethiopia, Hua2 from Peru, and two identical isolates (LSU-1 and LSU-3) from the United States (Tables 1 and 2).

amino acid identities were 97 to 100% and 96 to 100%, respectively. In these comparisons, the putative frameshift in the CP-encoding region of Ark15, starting from nucleotide 506 (base deletion) to 534 (base insertion), was ignored because it was suspected to be a sequencing error. When the respective base deletion and insertion were ignored, the putative frameshifted region of Ark15 was identical to those of several SPVG isolates (data not shown). Isolate Ark15 had an insertion of 3 nt (CAA) at position 528-530, whereas isolate Hua2 had a 6-nt insertion (TACAAA) at position 316-321, as compared to the other isolates of SPVG.

The CP-encoding region of the Chinese isolate Sichuan2 has been partially characterized for a relatively conserved 665-nt central portion that corresponds to the aa 109-325 in isolate Sichuan1 for which the complete CP sequence is available (Table 1). This part of the CP-encoding region showed only 88% nucleotide and 95% amino acid identity between these two isolates. Comparison of this 655-nt region in all five Chinese isolates (Henan, Sichuan1, Sichuan2, SPVG-CH, SPVG-CH2) revealed 86 to 99% and 94 to 99% identity at the nucleotide and amino acid levels, respectively (Table 3). These are the most divergent SPVG sequences compared.

Two recently deposited partial sequences (434 nt) of the CP-encoding region from two SPVG isolates detected in Spain (SPVGMa102 and SPVG181-3Can07) were available in GenBank (Table 1). These sequences began at the CP nucleotide position 269 and when compared to other SPVG CP nucleotide sequences (excluding the most different Chinese and Peruvian isolates), they were 98 to 100% identical (data not shown).

Phylogenetic analysis of SPVG CP-encoding sequences. To include the partial

CP-encoding sequences of the two isolates from the United States, phylogenetic analysis was carried out on only the N-proximal 612 nt of the CP-encoding sequences to determine whether the SPVG isolates belonged to different genetic lineages (Fig. 1A). Neighbor-joining analysis indicated that isolates SP1, JAAC, HAWA2, 3900A, CTB3, and Zimb1H formed one group, whereas the isolates Zimb1C, 3817, and PS1B formed another group (Fig. 1A). The two U.S. isolates differed from the Egyptian isolate Eg by only two nucleotides and formed a third cluster. The Chinese isolates CH and Sichuan1 and the Ethiopian isolate Ark15 were very closely related to each other, as were the isolates Henan and NZ-4 from China and New Zealand, respectively. The Chinese isolate SPVG-CH2 and the Peruvian isolate Hua2 formed a cluster distinct from all others (Fig. 1).

Phylogenetic analysis was also done using the central part (665 nt) available from isolate Sichuan2 and the corresponding part of all other isolates except those from the United States and Spain. Results indicated that in the main cluster including all but three SPVG isolates, the isolates Zimb1C, 3817, and PS1B formed, again, a cluster of their own, and also that Eg was distinguished from the others (Fig. 1B). Sichuan2, Hua2, and CH2 and all the other isolates represented four distinguishable genetic lineages or strains, respectively (Fig. 1B).

Additional verification of the sequences. Samples that were positive for SPVG when tested with the primers designed for SPVG were also positive when degenerate primers PVD-2 and 10820R that detect many different potyviruses were used. Amplification products were of the expected size, and the CP sequences of products obtained with both PCRs were

identical (data not shown). Also, the CP-encoding regions of isolates SP1 and SP2 from New Zealand (detected in post-entry quarantine in Perth, Australia) were cloned and sequenced independently in Finland and Australia, with identical sequence results in the two laboratories (data not shown). The CP-encoding sequences of the SPVG isolates from Hawaii, Java, and New Zealand were determined twice. The first time, the amplification products were cloned and sequenced, whereas the second time they were sequenced directly without cloning. Results from both analyses were identical (data not shown). These data show that the sequences of different isolates were genuine and not due to PCR or sequencing errors. One factor possibly contributing to accuracy in sequence analyses was the use of the DNA polymerase Phusion, which shows a 25-fold higher fidelity than the *Taq* DNA polymerase (6).

DISCUSSION

The results from this study together with those of other authors indicate that SPVG occurs in most sweetpotato production areas worldwide. Here, SPVG was found for the first time in parts of greater Oceania that represent the geographical extremities of the region, including Java, New Zealand, Hawaii, French Polynesia (Tahiti and Tubuai), and Easter Island, and also in southern parts of Africa in Zimbabwe and South Africa. Previously, SPVG was found in eastern Asia in China (11,12,19), northern Africa in Egypt (21), eastern Africa in Ethiopia (1), and North America in the southern United States (36). Recently, SPVG was also detected for the first time in South America in Peru (40) and in Europe in Spain (GenBank accession nos. EF577438 and EF577439). Therefore, this virus is now known to exist

Table 3. Percent nucleotide (upper diagonal) and amino acid (lower diagonal) sequence identities of the central part of the coat protein (CP) encoding sequences of *Sweet potato virus G* beginning from the nucleotide and amino acid positions 320 and 109, respectively

Isolate	Origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 SPVG-Eg	Egypt	***	99	97	98	98	98	98	98	99	99	99	99	99	99	99	88	86	86	80
2 Ark15 ^a	Ethiopia	99	***	98	99	99	99	99	99	99	99	99	99	99	99	99	89	87	86	79
3 SPVG-CH	China	97	98	***	98	98	98	98	98	98	98	98	98	98	98	98	87	86	86	79
4 Sichuan1	China	99	100	98	***	99	99	99	99	99	99	99	99	99	99	99	88	87	86	79
5 PS1B	Easter Island	99	100	98	100	***	100	99	98	99	99	99	99	99	99	99	88	87	86	80
6 Zimb1C	Zimbabwe	99	100	98	100	100	***	99	98	99	99	99	99	99	99	99	89	87	86	80
7 3817	Tahiti	99	100	98	100	100	100	***	99	99	99	99	99	99	99	99	88	87	86	79
8 Henan	China	98	99	97	99	99	99	99	***	99	99	99	99	99	99	99	88	86	86	79
9 NZ-4	New Zealand	98	99	97	99	99	99	99	99	***	99	99	99	99	99	99	88	87	86	79
10 JAAC	Java	99	100	98	100	100	100	100	100	99	***	100	100	100	100	100	89	87	86	79
11 Zimb1H	Zimbabwe	99	100	98	100	100	100	100	99	99	100	***	100	100	100	100	89	87	86	79
12 SP1	PEQ Australia	99	100	98	100	100	100	100	99	99	100	100	***	100	100	100	89	87	86	79
13 3900A	Tubuai	99	100	98	100	100	100	100	99	99	100	100	100	***	100	100	89	87	86	79
14 HAWA2	Hawaii	99	100	98	100	100	100	100	99	99	100	100	100	100	***	100	89	87	86	79
15 CTB3	South Africa	99	100	98	100	100	100	100	99	99	100	100	100	100	100	***	89	87	86	79
16 Sichuan2	China	94	95	94	95	95	95	95	95	95	95	95	95	95	95	95	***	87	86	76
17 Hua2	Peru	95	96	94	96	96	96	96	96	96	96	96	96	96	96	96	95	***	87	77
18 SPVG-CH2	China	95	96	94	96	96	96	96	95	95	96	96	95	96	96	96	94	94	***	77
19 SPV2		88	87	86	87	87	87	87	87	87	87	87	87	87	87	87	87	85	86	***

^a A putative frameshift in the CP-encoding region of Ark15 (nt 506-534) (1) was anticipated to be a sequencing error and was not taken into consideration in the deduced amino acid sequence.

Fig. 1. Phylogenetic analysis of partial coat protein (CP) coding sequences of *Sweet potato virus G* (SPVG) isolates. Isolates marked with an asterisk (*) were characterized in this study. **A**, Analysis of the N-proximal 612 nt of 19 isolates. The C-proximal part was omitted because it is not available from the two American isolates (LSU-1 and LSU-3). **B**, Analysis of the central part (665 nt) of the CP encoding region starting from position 320, including the Chinese isolate Sichuan2, of which only the sequence of this region is available. Isolates LSU-1 and LSU-3 were excluded. The CP sequence of Sweet potato virus 2 (SPV2), isolate Aus55-4 (GenBank accession no. AM050885) was used as an outgroup. The bootstrap values exceeding 60% of 1,000 replicates are shown. Scale indicates the Kimura nucleotide distance (Knucl).

in most regions of the world where sweet-potato is grown as a major crop for subsistence, as a cash crop, or for industrial purposes.

This study revealed the presence of a surprisingly homogenous global population of SPVG isolates. The exceptions were two isolates from China (SPVG-CH) (12) and the Peruvian isolate Hua2 (40), which each differ clearly from all others. Considering the length of time sweetpotato has been distributed around the world and the separate distribution routes it has taken, this result was unexpected and is intriguing, as much greater sequence divergence might have been expected. Currently, it appears that two isolates from China, one isolate from Peru, and all the rest represent four distinct genetic lineages of SPVG. Studies of genetic diversity in populations of the two other sweetpotato-infecting potyviruses, SPV2 and SPFMV, for which a similar or larger number of isolates has been characterized, revealed a similar picture. In SPV2, genetic diversity of the CP-encoding sequences is within the range of that for SPVG, and SPV2 isolates form at least three distinct phylogenetic groups (37). However, data are insufficient to conclude whether any strain of SPV2 shows a worldwide distribution similar to that of SPVG. Isolates of SPFMV can be placed into four phylogenetic groups, of which one (EA) is largely but not entirely restricted geographically to East Africa (39). Isolates belonging to the other groups are distributed worldwide (39). The CP-encoding region of strain C of SPFMV is phylogenetically distant from the other three strains which are closely related and, additionally, contains a 6-nt deletion (39). Data from this study indicated that the CP in isolates of SPVG can have at least three lengths due to insertion of one (Ark15) or two codons (Hua2), as compared to other isolates.

Sequence differences between the Chinese and Peruvian SPVG isolates show that considerable sequence divergence exists, and yet, there was relatively little evidence of this in isolates collected from the rest of the world. The limited sequence divergence of isolates characterized in this study suggests the same SPVG strain consisting of minor sequence variants (i.e., a viral quasispecies) (14) was spread widely via exchange of sweetpotato germplasm, by plant breeding activities, or through the international trade of sweetpotatoes for use as a staple food. It seems noteworthy that in this study, sweetpotatoes purchased from farmers' markets in several countries in distant locations were found to be SPVG infected. Sweetpotatoes maintain viability during long-distance transport (as observed in this study), and can produce SPVG-infected foliage acting as a source of virus in the new geographic location. Hence, increased global movement of plant materials may have resulted in in-

creased dispersal of a homogenous SPVG population around the world. Alternatively, SPVG might be unusually stable or the divergent strains less fit. Such possibilities cannot be excluded until more studies have been undertaken with SPVG.

An interesting task for future studies would be to determine the center of diversity and evolutionary dispersal of SPVG. At present, significant genetic variability of SPVG is known only in China. It may simply be that insufficient studies have been carried out elsewhere. On the other hand, it is intriguing that five isolates from China exhibit larger genetic variability than is found among 17 isolates characterized from all other continents. It is uncertain whether sweetpotato was introduced to China from the Philippines after Columbian times, or whether the Treasure Fleets of Cheng Ho (Zheng He) in 1405-1435 or previous voyages by other Chinese sailors to wide areas of the Pacific Ocean and the Caribbean over hundreds of years in the pre-Columbian times (31) introduced the new crop from these areas, where it was grown at that time (32). Taken together, it seems likely that movement of sweetpotatoes has occurred in the Pacific region for a long time (10,32). Future studies will show whether SPVG originated in the center of origin of the crop in Mexico and Central America (42) or in China, as both seem possible in light of the current information.

This study indicated that SPVG may be difficult to detect in many sweetpotato cultivars or landraces because infected plants rarely display symptoms and virus titers are too low to be detected by serological tests. Graft-transmission of the virus from sweetpotatoes to *I. setosa* is therefore recommended to enhance SPVG titers to levels detectable by serological assays, preferably employing DAS- or TAS-ELISA. RT-PCR was found to be more sensitive than either but is not yet routinely used for sweetpotato virus diagnostics in most laboratories. In this study, SPVG was detected in cv. Owairaka Red from New Zealand in post-entry quarantine in Western Australia by indexing on *I. setosa* and by DAS-ELISA. SPVG and SPFMV co-infecting the plants were subsequently successfully eradicated by heat treatment and meristem tip culture. The original material was destroyed and a healthy clone released to the importer. Taken together, this suggests that symptomless infections could be a major problem that inadvertently enables the movement and distribution of SPVG from infected to uninfected regions. This is not a problem specific to SPVG, as in our experience other potyviruses such as SPFMV and SPV2 also often infect sweetpotato cultivars without apparent symptoms. For this reason, international quarantine measures for sweetpotatoes should be enhanced, and testing of imported germplasm, for all

viruses of sweet potatoes, should become routine.

There is little data on the economical importance of SPVG. The lack of symptoms in SPVG-infected plants found in this study also was noted by others (26). In a previous study, SPVG or SPFMV alone caused no significant yield loss in sweetpotato cv. Beauregard, whereas co-infection with these two viruses reduced the yield by 14% (9). The yield effects of SPVG and also of many other potyviruses may have been overlooked in sweetpotatoes because SPFMV and its negative impacts dominate in all sweetpotato growing areas of the world (23,39).

Co-infection of SPCSV with SPVG was observed to enhance SPVG titers and symptom severity in cv. Beauregard in a previous study (26). Elevated SPVG titers were also found in this study in landrace sweetpotato from French Polynesia experimentally co-infected with SPVG and SPCSV. In areas where both SPFMV and SPCSV occur, their co-infection causes the very severe sweetpotato virus disease (SPVD) (39), which again is likely to mask any effects of SPVG and lead to the general assumption that yield losses are solely caused by SPFMV and SPCSV. In conclusion, the incidence of SPVG in individual sweetpotato crops and the disease and growth effects it may have on different sweetpotato cultivars both warrant further investigation.

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